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Oral treatment of ichthyosis by the cytochrome P-450 inhibitor liarozole

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Summary Liarozole, a novel imidazole derivative, inhibits the cytochrome P450-dependent 4-hydroxylation of retinoic acid, resulting in increased tissue levels of retinoic acid. Twelve male patients with ichthyosis were given oral liarozole, 150 mg twice daily, in an open study for 12 weeks. Immunohistochemical parameters of inflammation, epidermal proliferation and differentiation were assessed before and after treatment. Extent and severity of the skin lesions was markedly reduced in all patients. Clinical side-effects were reminiscent of those with synthetic retinoids. No relevant changes were found in the haematological, urinary and biochemical parameters. Immunohistochemical assessment showed a statistically significant induction of keratin 4 after liarozole treatment in 10 of 12 patients. In two of these patients keratin 13 was induced. This open study showed that oral liarozole treatment was efficacious and well tolerated in the treatment of different types of ichthyosis. The immunohistochemical results suggest a retinoid mechanism as the mode of action.

Retinoids (vitamin A–acid derivatives) are important compounds that control proliferation and differentiation of epithelial tissues in mammals.[1–4] Accordingly, systemic treatment with retinoids has yielded good results in the treatment of disorders of keratinization such as ichthyosis. However, their toxicity and teratogenicity limit their use to severely affected patients.[5–8] Therefore, a search for new drugs with a broader toxic–therapeutic window is indicated.

Liarozole, a novel imidazole derivative, inhibits the cytochrome P450-dependent 4–hydroxylation of retinoic acid which is the physiological breakdown of all-*trans*-retinoic acid. In rats, liarozole increases the concentration of retinoic acid in plasma and tissue.[9] Liarozole also inhibits several cytochrome P450-dependent steps in steroid biosynthesis, mainly the conversion of androgens to oestrogens (aromatase), progestins to androgens (17-hydroxylase, 17 -20-lyase) and of 11-deoxycorticosterone to corticosterone (11-hydroxylase).[10] Administration of 150 mg liarozole twice daily for 10 days to male volunteers showed neither reduction in cortisol levels, nor in the cortisol surge after adrenocorticotrophin hormone stimulation.[11] Apart from a transient decrease of testosterone levels, no changes of the levels of this hormone were observed. Furthermore, no abnormalities were observed in cortisol, cholesterol and testosterone levels in psoriasis patients, treated with doses ranging from 75 to 150 mg twice daily.[12] One open trial with liarozole, 75–150 mg twice daily, in severe plaque psoriasis, showed a significant improvement in 77% of the patients.[12] Evidence is accumulating that liarozole will provide a broad efficacy side-effect window. However, final conclusions await further comparative studies.

The aim of the present study was to investigate the effectiveness and tolerability of oral liarozole in 12 patients with hereditary ichthyoses. Additionally, to gain insight into the *in vivo* action of liarozole on these disorders, immunohistochemical parameters of inflammation, epidermal proliferation and differentiation were assessed before and after treatment.

Patients and methods

In an open prospective study, 12 male patients suffering from various forms of ichthyosis were included: five with X-linked recessive ichthyosis (XRI), four with non-erythrodermic lamellar ichthyosis (NELI), one with erythrodermic lamellar ichthyosis (ELI) and two with bullous congenital ichthyosiform erythroderma

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(BCIE). They were treated with oral liarozole (liarozole fumarate), 150 mg twice daily, for 12 weeks. Topical as well as systemic treatments were stopped 2 and 4 weeks before the start of the study, respectively. Patients were allowed to use bland emollients during the study except on the days of the visits. Only male patients aged 18–70 years, were included. The patients with XRI had a documented steroid sulphatase deficiency, and sun exposure had to be avoided. Patients were examined before treatment, and at 0, 1, 2, 4, 8 and 12 weeks during the treatment phase, and 4 and 8 weeks after discontinuation of therapy. The severity of involvement was rated using a four-point scale for scaling, roughness, erythema, bullae and hyperpigmentation, as follows: 0, absent; 1, mild; 2, moderate; 3, severe. Scaling and roughness were recorded in all subgroups, erythema and bullae in ELI and BCIE, and hyperpigmentation only in XRI. The extent of involvement was expressed as the percentage of affected skin on arms, trunk and legs. An overall response to treatment was quantified by both the investigator and the patient, on a five-point scale: 0, deteriorated; 1, unchanged; 2, moderately improved; 3, markedly improved; and 4, cleared.

Haematological (haemoglobin, haematocrit, red blood count, white blood count and differential, platelet count), biochemical (Na, K, Cl, Ca, P, urea, creatinine, albumin, protein, total bilirubin, cholesterol, triglycerides, alkaline phosphatase, transaminases and glucose) and urinary analysis (specific gravity, pH, protein, glucose, urobilinogen, bilirubin, red blood count and white blood count) were performed at entry, and at each visit after start of treatment. Statistical analysis was carried out using the Wilcoxon signed-rank test for paired data.

In all 12 patients, punch biopsies were taken from representative skin lesions before and after treatment. The antibodies used to assess epidermal proliferation, keratinization, and epidermal and dermal inflammation are summarized in Table 1. Immunohistochemical procedures were carried out as described previously.[13]

Results

Severity of skin involvement, assessed by the investigator, is presented in Figure 1. For the extent of involvement, a comparative curve was observed. As expected, the extent and severity of the ichthyosis deteriorated during the wash-out phase and, the severity, even during the first week of treatment. Thereafter, a clear clinical improvement for both the extent and severity of the skin lesions was achieved. At the end of the treatment phase, an impressive clinical improvement was achieved in all patients (Figs 2 and 3). The extent of skin lesions was statistically significantly reduced for arms ($P < 0{\cdot}001$), trunk ($P = 0{\cdot}002$) and legs ($P < 0{\cdot}001$). The degree of scaling and roughness, also significantly improved on the arms ($P < 0{\cdot}001$), trunk ($P = 0{\cdot}001$) and legs ($P = 0{\cdot}001$). As hyperpigmentation, erythema and bullae were only assessed in a few patients (5, 1 and 2, respectively), no statistics were carried out. Nevertheless, changes in hyperpigmentation were comparable with changes in roughness and scaling. Erythema was not remarkably influenced. For overall response, both the investigator and 11 patients assessed the treatment as a marked improvement. One patient reported a moderate improvement. In six patients, after discontinuation of the treatment, the extent and severity of the lesions gradually worsened to reach

Table 1. Specificity and sources of the antibodies

Antibody	Specificity	Source
Proliferation		
Ki-67	Nuclear antigen	Dakopatts, Copenhagen, Denmark
Keratinization		
Ks8.12	Keratin 13 and 16	Sigma, St Louis, U.S.A.
RKSE60	Keratin 10	Eurodiagnostics, Apeldoorn, the Netherlands
6B10	Keratin 4	Eurodiagnostics, Apeldoorn, the Netherlands
1C7	Keratin 13	Eurodiagnostics, Apeldoorn, the Netherlands
Inflammation		
Anti-elastase	Elastase (polymorphonuclear leucocytes)	Serotec, Oxford, U.K.
T11	CD2 (T lymphocytes)	Dakopatts, Copenhagen, Denmark
WT14	CD14 (monocytes, macrophages)	Department of Nephrology, Nijmegen, the Netherlands
OKT6	CD1a (Langerhans cells)	Ortho Diagnostics Systems, Raritan, U.S.A.

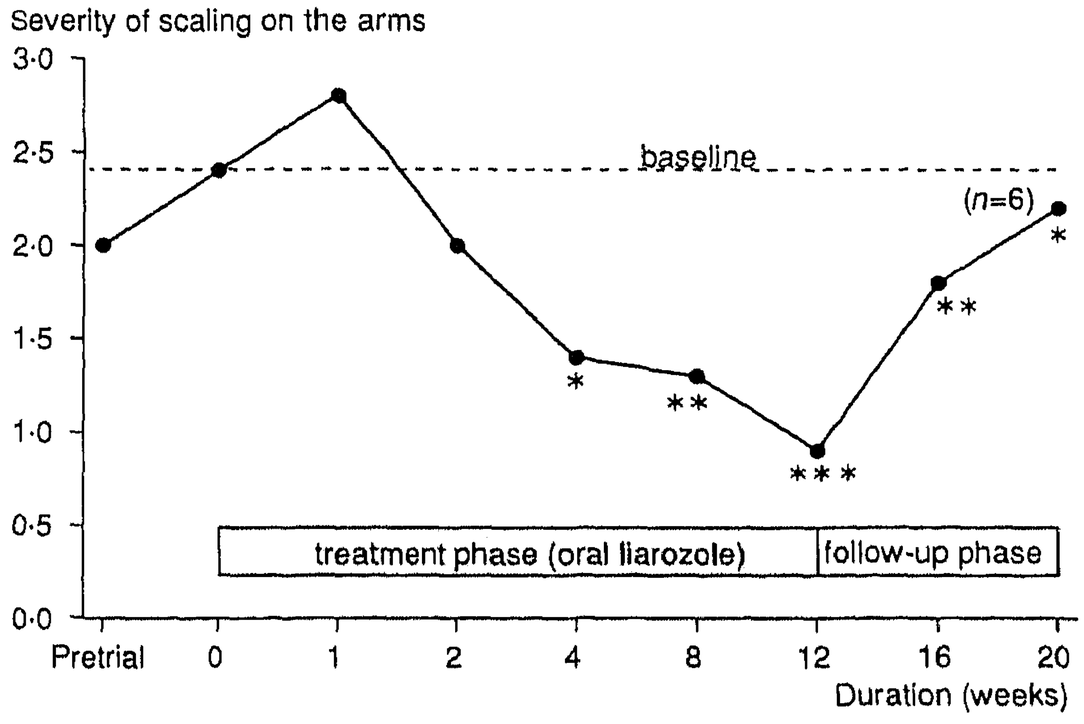

Figure 1. Clinical severity. The severity of scaling on the arms worsens during the wash-out period and the first week of treatment. Afterwards, a gradual improvement, reaching its maximum at week 12, is observed. Discontinuation of the treatment is followed by progressive worsening of this symptom to pretreatment values at week 8. Whereas all 12 patients were evaluated until week 16, only six of them were considered at week 20 because the others required treatment. For the severity of scaling on trunk and legs, as well as the severity of roughness and hyperpigmentation on arms, trunk and legs, a similar pattern is observed. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}005$, $^{***}P < 0{\cdot}001$.

pretreatment values after 8 weeks. The other six patients already required treatment after 4 weeks.

Eleven patients experienced adverse events. The most frequently reported clinical side-effects were dry lips (10 patients), itching (six) and dry eyes (three). These side-effects were mild, not requiring discontinuation of treatment in any patient. Of the two patients who mentioned hair loss, one improved during treatment and the other patient improved upon discontinuation of

Figure 3. (a) A patient with congenital bullous ichthyosiform erythroderma before treatment, showing thick yellowish scales over the neck, on an erythematous skin. (b) At the end of the treatment phase, a substantial reduction of scales is observed. Some erythema is still present.

treatment. One patient who had BCIE developed multiple large bullae on a dose of 150 mg twice daily, but these disappeared upon reduction of the dose to 150 mg daily. One patient with XRI developed urticaria after 2 weeks of treatment. After a temporary stop of 2 weeks the lesions did not reappear, and no relapse occurred after gradual readministration of liarozole, first 150 mg daily for 2 weeks, followed by the initial dose of 150 mg twice daily. Dryness of the nose, headache, gastric pain, increased skin tension, burning skin sensation, dizziness, enhanced sweating, candidiasis and undefined erythema were each observed in one occasion. Haematological, biochemical and urinary parameters, measured at week 12, were not significantly influenced by liarozole, compared with the pretrial visit.

The most obvious histochemical effect was seen for the expression of keratin 4 (Table 2). While keratin 4 was absent at baseline, it was expressed in 10 of the 12

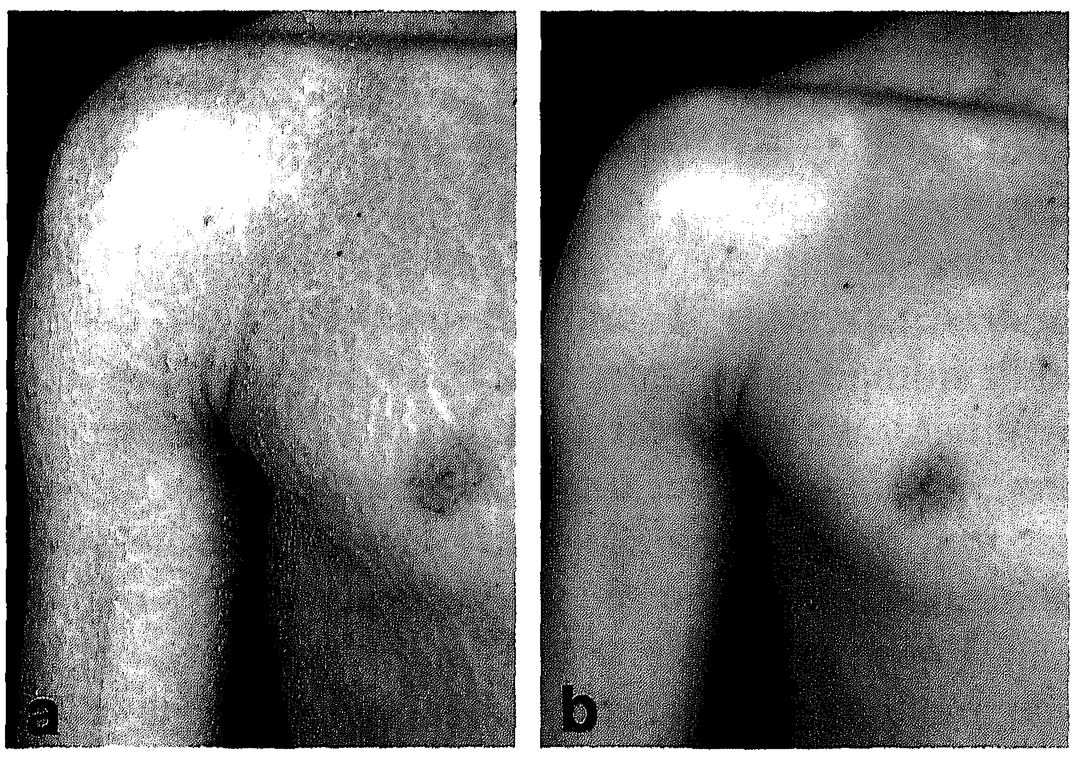

Figure 2. (a) A patient with non-erythrodermic lamellar ichthyosis before treatment, displaying large brown-coloured scales, covering a substantial part of the body. (b) After 12 weeks of treatment, a marked reduction of scaling is observed, resulting in a normal appearance.

Table 2. Modulation of expression of keratins 4, 13 and 16, in the various ichthyosis subgroups, under influence of liarozole therapy

Parameter	X-linked recessive ichthyosis ($n = 5$)	Non-erythrodermic lamellar ichthyosis ($n = 4$)	Erythrodermic lamellar ichthyosis ($n = 1$)	Bullous congenital ichthyosiform erythroderma ($n = 2$)
Keratin 4 (6B10)				
unchanged	1	1	0	0
increased	4	3	1	2
Keratin 13 (1C7)				
unchanged	5	4	0	1
increased	0	0	1	1
Keratin 13/16 (Ks8.12)				
reduced	0	0	1	0
unchanged	1	3	0	1
increased	4	1	0	1

patients after 12 weeks of treatment, independently of the type of ichthyosis. The induction of keratin 4 was statistically significant ($P < 0{\cdot}05$). Keratin 13 could not be detected at baseline, but was sporadically detected in two patients after 12 weeks of treatment. The expression of keratins 13 and 16 was variable: increased in six patients, reduced in one and inconsistent in five. Treatment with liarozole did not change the baseline expression of keratin 10 which could be detected within the suprabasal compartment. Before treatment, the average number of actively cycling cells (monoclonal antibody Ki-67) was 88·9 per mm of section. After 12 weeks of treatment, this number was 86·6 ($P < 0{\cdot}10$). None of the inflammatory parameters were significantly changed with treatment.

Discussion

Twelve patients with different types of ichthyosis, showed a statistically significant improvement in the extent and severity of the skin lesions after 12 weeks treatment with liarozole. As anticipated clinical side-effects were consistent with those experienced in hypervitaminosis A, i.e. mostly dry lips, dry eyes and itching. These effects were mild and did not necessitate discontinuation of the treatment. No significant changes were observed in the haematological, biochemical and urinary parameters. The present results suggest that liarozole provides a substantial clinical efficacy with limited side-effects.

The treatment of disorders of keratinization has been revolutionized by systemic retinoids.[14] However, the margin between safety and efficacy is limited for these drugs. Based on 12 patients, it is virtually impossible to conclude whether the therapeutic window between efficacy and side-effects is broader for liarozole compared with acitretin. However, the present study suggests that liarozole is at least as effective, whereas the side-effect profile is rather similar to that of acitretin. In particular, the mucocutaneous side-effects that occur within the therapeutic dose range, is a characteristic for both treatments. Furthermore, dose-dependent blistering occurs both during liarozole and acitretin treatment in BCIE.[15] In our opinion, a double-blind comparative study between liarozole and acitretin is warranted in patients with ichthyosis. The development of a topical liarozole formulation could help to broaden the margin between efficacy and side-effects even more.

Although the clinical response in various disorders of keratinization, and mucocutaneous side-effects, suggest that liarozole might work via a retinoid effect, further evidence for such an effect might be provided by immunohistochemical studies on the cell biological effects during treatment. Immunohistochemical assessment of biopsies revealed a statistically significant induction of the expression of keratin 4 in 10 of 12 patients ($P < 0{\cdot}05$). In two patients, keratin 13 was induced. Keratins 4 and 13 are absent in normal adult human skin. Induction has been described after treatment with topical retinoids.[16,17] Therefore, the present results provide indirect evidence for the hypothesis that the pharmacological effects of liarozole could be mediated by enhanced levels of endogenous retinoic acid. However, further studies on retinoid levels in the skin and induction of expression of cellular retinoic acid binding protein II are required before this hypothesis can be proven.

Theoretically, topical application of liarozole may be effective too. However, at the start of this study topical

liarozole was not available. Furthermore, topical retinoids used in the past were characterized by a high irritancy potential.[18] Based on the good clinical results obtained with oral liarozole in this study, a topical liarozole formulation has been developed. Studies on topical liarozole in ichthyosis are ongoing.

In conclusion, in this open study, treatment with liarozole was followed by a substantial clinical improvement in all patients with ichthyosis. Relevant laboratory side-effects were absent. Subjective side-effects were reminiscent of those observed with synthetic retinoids. The immunohistochemical results lend further support for a retinoid mechanism, although other modes of action are not excluded. Additional controlled studies are required to confirm the efficacy and to determine optimum dosage and safety profile of this new compound.

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